

## Evaluation of the PANBIO *Brucella* Immunoglobulin G (IgG) and IgM Enzyme-Linked Immunosorbent Assays for Diagnosis of Human Brucellosis

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**PANBIO *Brucella* immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays (ELISAs) were assessed against *Brucella* standard agglutination tube and Coombs tests. The sensitivities of ELISA IgG and IgM were 91% and 100%, respectively, while the specificity was 100% for both. These ELISAs are simple, rapid, and reliable for the diagnosis of human brucellosis.**

Brucellosis remains a prevalent disease in humans and animals in many countries around the world, especially those in the Middle East and the Arabian Gulf (2, 7). The clinical features and presentation of human brucellosis overlap with many other infectious and noninfectious diseases (13). Therefore, its accurate diagnosis necessitates the use of specific tests, mainly culture and serologic tests (1).

Several serologic tests have been developed for the diagnosis of human brucellosis, including the standard agglutination tube (SAT) test, anti-human globulin (Coombs) test, indirect fluorescence antibody (IFA) test, and enzyme-linked immunosorbent assay (ELISA) (3, 12, 16). SAT is the primary test used in many clinical laboratories. Although tests such as IFA and ELISA are simple and reliable for the detection of immunoglobulin (Ig) classes especially in complicated cases (3, 9, 14, 16), many laboratories still use the classical Coombs test, as an extension of SAT, to detect “incomplete,” “blocking,” or “non-agglutinating” anti-*Brucella* antibodies, such as IgG (8, 10, 12).

Comparative studies among tests have shown the superiority of ELISA in detecting chronic and complicated cases of brucellosis. However, most of the previously reported ELISA techniques used were developed in-house (4, 6, 15).

This study was undertaken to evaluate commercial *Brucella* IgG and IgM ELISA kits (PANBIO, Windsor, Brisbane, Australia) in comparison with SAT and Coombs by using sera from patients with brucellosis and controls.

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Sixty-five consecutive sera submitted for *Brucella* serodiagnosis, each from one patient, showing positive titers by the *Brucella* SAT test and/or the anti-human globulin test (Coombs), were included in this study. In addition, 68 sera from apparently healthy individuals, showing negative SAT and Coombs tests, and from patients with positive findings for

autoimmune markers and for several bacterial and viral diseases were included as controls.

The SAT test was performed on serum dilutions of 1:20 to 1:1,280 by using *Brucella abortus* antigen (Immunostics, Inc., N.J.), as previously described (12). The anti-human globulin (Coombs) test was performed, as an extension of SAT, for detection of “incomplete,” “blocking,” or “nonagglutinating” IgG antibodies, as previously described (12), by using anti-human globulin reagent (anti-IgG; Ortho Diagnostic Systems, N.J.). Positive results were defined as any sample showing agglutination with SAT and/or Coombs at any level. The results were available after 24 and 48 h for SAT and Coombs testing, respectively.

The PANBIO *Brucella* IgG and IgM ELISAs were performed and interpreted according to the manufacturer's instructions (PANBIO, Windsor, Brisbane, Australia). Each run included positive, negative, and cutoff calibrator controls. An index value (PANBIO units) was calculated to generate the results for either IgG or IgM as follows: negative, <9; equivocal, 9 to 11; and positive, >11. The ELISAs could be completed in around 2.5 h.

The assay results for the 65 sera from patients with suspected brucellosis tested by the different methods were divided into four groups (I to IV) based on serological profiles, as shown in Table 1.

Overall concordant results between ELISA IgG and ELISA IgM titers, and between SAT and Coombs titers, were found among 91% of the *Brucella* patient sera (groups I to IV). Six samples yielded discrepant results: these were positive by SAT, Coombs, and *Brucella* ELISA IgM titers but showed negative *Brucella* ELISA IgG (group IV). This could either indicate a false-negative ELISA IgG or a false-positive Coombs. Alternatively, these results may represent ELISA IgM false positives and ELISA IgG false negatives. All control sera showed negative results in all tests. The sensitivities of *Brucella* ELISA IgG and IgM were 91% and 100%, respectively, while the specificity was 100% for both.

The SAT and Coombs serologic tests used in this study are relied upon most frequently for the diagnosis of brucellosis. In this comparative study, the PANBIO ELISA kits showed con-

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TABLE 1. Distribution of *Brucella* antibody findings among 65 patients with brucellosis tested by different methods

<i>Brucella</i> group <sup>a</sup>	Test result for:				No. (%) of sera
	SAT	Coombs	ELISA		
			IgG	IgM	
I	+	+	+	+	46 (71)
II	—	+	+	—	7 (11)
III	+	+	+	—	6 (9)
IV	+	+	—	+	6 (9)

<sup>a</sup> Divisions are based on serological profiles.

cordant results with the SAT and Coombs assays and can thus be reliably used for the diagnosis of human brucellosis. A discussion on the advantages and drawbacks of each of these tests is briefly warranted, as they were detailed in an earlier review (1).

The agglutination tests in tubes, e.g., SAT, or on slides (Rose Bengal) continue to be the mainstay of laboratory diagnosis, due to their simplicity, low cost, and reliability (>90% sensitivity) in diagnosing acute brucellosis. In addition, agglutination tests have been helpful in monitoring a noncomplicated course of acute brucellosis. However, SAT and the other formats of direct agglutination tests suffer from high false-negative rates in complicated and chronic cases (1, 13).

An extension of SAT is the indirect *Brucella* Coombs test. Generally, the latter is more reliable than SAT in detecting anti-brucella antibodies especially when IgG alone is present in the tested sera. The Coombs test is used to detect nonagglutinating or incomplete antibodies (8, 10, 12). This test can also best serve laboratories that do not perform ELISA when they are faced with a SAT-negative suspected brucellosis case, such as in complicated and chronic cases. However, Coombs was also reported to suffer from false-negative results (8, 10, 14), and in our experience it can miss around 7% of cases (6).

Other drawbacks of Coombs include the fact that it is labor-intensive, since centrifugation and washing of the pellet is time-consuming, especially if more than one serum is to be tested, and the fact that result interpretation is subjective (11, 12). Moreover, “incomplete” antibodies are not always detected by Coombs (6, 10). Because of the limitations of the Coombs test, other assays, such as IFA and ELISA, that can reveal the classes and subclasses of immunoglobulins in a sensitive and simple manner were sought. Although there are a few comparative studies between ELISA and Coombs, none has been performed for cases of neurobrucellosis, and in our current study we did not encounter such cases for comparison. However, in our experience ELISA has proved to be very reliable in diagnosing such cases (1, 3, 6, 7, 15).

Concerning ELISA, several studies have shown that it is the test of choice for the diagnosis of complicated and chronic cases, especially when other tests are negative (1, 3, 5, 13).

Moreover, this assay reveals total and individual specific immunoglobulins rapidly (within 3 to 5 h) and reliably. In addition, ELISA performance surpassed the other tests in the diagnosis of chronic and complicated cases such as patients with neurobrucellosis (4, 5, 7, 15). However, it cannot be advocated for routine use in the diagnosis of patients with acute brucellosis since the agglutination tests for this condition are as reliable and less expensive than ELISA (1, 16).

In conclusion, the PANBIO *Brucella* ELISA showed concordant results with SAT and Coombs tests and can be reliably used for the diagnosis of human brucellosis. As noted in the literature, ELISA also provides all the advantages of Coombs in a simpler and more reliable way and bears a better relation to clinical findings. Thus, ELISA in general is considered and recognized as the test of choice in case of clinical suspicion of brucellosis, even when the Coombs test is negative.

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